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CELL AND NUCLEAR DIVISION IN FULIGO VARIANS.

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(WITH PLATE XIV)

OUR knowledge of such important vital phenomena of the Myxomycetes, as the formation of the spores and the capillitium, and nuclear and cell division, is still based quite largely on the data given in the single paper published by Strasburger (**23**) in 1884.

Strasburger studied *Trichia fallax*, and his main conclusions were that the nuclei divide karyokinetically just before spore formation, the capillitium is formed in vacuoles, and the spores are formed by simultaneous breaking up of the multinucleated mass along hyaline lines into uninucleated spores. Strasburger found his material on decaying stumps, and speaks of finding all stages in the development of the sporanges simultaneously. Lister mentions also that the sporangia require several days to mature after their first appearance.

Strasburger describes the capillitium, which in *Trichia fallax* consists of long spirally-thickened threads tapering at each end, as arising in vacuoles of the protoplasm. These vacuoles become elongated, and the hollow tubular capillitium thread is formed in their interior. Strasburger describes the wall of the thread as being formed by the fusion of microsomes, which become aggregated in the membrane of the vacuole, and unite to form a thin, transparent pellicle. Further deposition of

microsomes in spiral lines about the so formed thread leads to the formation of the spiral ridge-like thickenings of the mature capillitium. The whole process is identical with that which takes place in the formation of a cell wall, according to Strasburger's earlier accounts of that process.

Whether or not the microsomes are the units in cell wall formation, we have here excellent evidence that the interior protoplasmic surface, which lies next to the vacuole, is equivalent to the exterior surface which forms the peridium of the entire sporange. Each surface is able in essentially similar fashion to deposit on occasion a resistent membrane over its whole extent. The doctrine of the equivalence of plasma-membrane and vacuolar membrane as developed by Pfeffer and De Vries finds strong support in this method of capillitium formation. Strasburger describes a period of nuclear division as preceding spore formation. The division is karyokinetic and the equatorial plate, separation of the daughter chromosomes, and development of the daughter nuclei are figured. The spindle fibers are inclined only slightly toward each other at the poles. The nuclei all divide at the same time, so that each section shows thousands of karyokinetic figures. Strasburger says little as to the method of spore formation. He figures the cleavage as producing the one-nucleated spores directly, and describes the boundaries of the spores as at first consisting of granules and then of clear lines, and notes also that the differentiation proceeds for the most part from the periphery toward the center. The young spore is at first polygonal, then rounds itself up and becomes enclosed by a wall.

Zopf (**27**) adheres to the view that the capillitium represents plasma masses (Hyaloplasma, Gerustplasma) which are not used for spore formation and have become hardened. He does not pretend, however, to have verified this statement for himself. Later (p. 63) he accepts Strasburger's account of the formation of the capillitium in Trichia, and is inclined to regard it as true for all the forms with hollow capillitia (Coelonemata), while still holding that the forms having a capillitium composed

of solid threads (Stereonemata) form the latter from strands of protoplasm. As a matter of fact, both forms of capillitium may arise in vacuoles, as I have been able to determine in the cases of Stemonitis and Lycogala. The account given by Strasburger is correct, and I have not thought it necessary to give further figures at this time. Whether the thread is hollow or solid, simple or branched, free or connected with the peridium or a columella, are entirely secondary conditions, depending on the extent and form of the vacuoles.

Massee neglects Strasburger's account of capillitium formation altogether, and advances the extremely loose and erroneous view that generally a surplus portion of the protoplasm takes the form of a more or less complicated network mixed with the spores, and homologous with the strands described as being present in the sporangium of Mucor, inasmuch as both structures are made from a substance separated from the protoplasm during spore formation. I have shown (4) that the so-called intersporal protoplasm of the Zygomycetes is merely excreted slime, and Strasburger's account of capillitium formation is true for all slime molds that have as yet been carefully investigated.

Lister has discovered karyokinetic division of the nuclei in the sporanges of a considerable number of genera, and has observed karyokinetic figures in the dividing swarm spores and in the growing plasmodia, though he also figures direct division as occurring at this latter stage. He concludes that whenever cell formation occurs in the life history of the Mycetozoa, the nuclei divide by karyokinesis. Lister also observed in certain genera lobed plasma-masses containing six to ten nuclei in the equatorial plate stage, and describes these masses as separating into uninucleated spores during the succeeding stages of nuclear division. For other genera he confirms the account of Strasburger, according to which nuclear division is complete before spore formation begins.

De Bary (1a) follows Strasburger in describing the spore formation for the whole group of slime molds as taking place by simultaneous breaking up of the protoplasm of the sporange

into uninucleated masses, and this is the current statement of botanical text-books.

Rosen (17) made a very thorough study, as he describes it, of *Fuligo septica*. He finds nuclei of two kinds, one poor in content and containing a so-called middle-body, and the other so densely filled with stainable substances as to appear almost homogeneous. The relative number of these two types of nuclei vary at different stages in the development of the slime mold. Nuclear division occurs prior to spore formation, but the process is described as much simpler than in the higher plants. Rosen thinks it belongs to the karyokinetic type, but it is doubtful whether a spindle figure is formed, etc. The cleavage is positively stated to be simultaneous, and to take place by the deposition of a network of granular plates which cut the protoplasm up at once into polyedric uninucleated spores. These plates are said to show microsomes very plainly, the latter being placed at right angles to the plane of the plate.

As will be seen below, my own observations on Fuligo have led to entirely different results from those of Rosen. I am convinced that his two forms of nuclei are due to inequalities in fixation such as sometimes occur. As to the method of nuclear division and spore formation I am certain that Rosen failed to find material in the stages when these processes occur. His description of nuclear division must have been based on resting nuclei whose contents happened to be somewhat unusually placed. As for the network of granular plates with microsomes such as he figures, I am convinced that no such structures are to be found in Fuligo at any stage of development. The difficulties in the way of obtaining accurate results in the study of fungus cells and nuclei are great, but not sufficient to justify such slipshod results as those of Rosen in the paper under consideration.

A summary of our present knowledge of the Myxomycetes has recently been published by Jahn (9), and reference may be made to it for a further account of the literature of the group.

My material was fixed in Flemming's solution, weaker formula, sectioned, and stained with Flemming's safranin, gentian-violet and orange.

The formation of the aethalium of Fuligo has been very well described by De Bary so far as its grosser structure is concerned. The plasmodium which is ready to form spores creeps to the surface of the substratum, and there forms a reticulum which is similar to that of the vegetative condition except that it is more dense. It becomes a rounded cake-like mass, the meshes of the reticulum being relatively small and the protoplasmic strands very thick. We have in fact at this stage a contracted reticulum, the interprotoplasmic spaces having become minute. At this stage the solids which have been imbedded in the protoplasm are all thrown out upon its surface. Large amounts of water containing salts in solution are excreted, and, the water evaporating, the salts are deposited as crystals along with the rejected solids. These waste materials are found in all the meshes of the protoplasmic reticulum, and form a sort of fragile framework piercing the ripe aethalium in all directions. The yellow coloring matter of the plasmodium is also transferred to these waste materials, so that the protoplasm is left apparently homogeneous and colorless.

A further step in forming the aethalium consists in the continued contraction of the protoplasmic reticulum, so that its superficial strands are withdrawn toward the center. In this withdrawal of the protoplasm from the peripheral parts of the mass the excreted wastes are left behind, and form thus a porous friable crust over the surface of the protoplasmic mass. At the margins of the aethalium this waste material frequently takes the form of a thin membranous almost papery border. In this further contraction of the protoplasmic reticulum the interprotoplasmic spaces become reduced in many cases to mere strands or plates of the yellow colored waste materials described above. In other cases the spaces remain as oval or angular lacunae lined with a thin yellow crust of the same excreta. *Fig. 1* shows a section through a portion of an aethalium relatively free from

such cavities. In *fig. 2*, from another portion of the same aethalium, parts of several lacunae are shown, and their relative size and distribution is thus partially indicated.

In addition to the solid particles and solutions thrown out, the protoplasm excretes over its whole surface a thin fragile membrane, in which the crystals of lime are frequently partly imbedded. This membrane is by no means as thick as in the case of the slime molds which produce sporanges, but it appears very clearly in microtome sections. In most regions it is hardly more than a cement to hold together the lime crystals in a continuous film. In other regions, where these are less abundant, it appears as a very thin homogeneous membrane. It lines all the interprotoplasmic cavities mentioned above, as well as covering the peripheral portions of the protoplasm. It is always next to the protoplasm itself. I have never found crystals or other solid excreta between it and the plasma membrane.

At a stage when cleavage is just beginning, such as that shown in *fig. 1*, the nuclei are generally in the resting condition, and are distributed rather unevenly through the cytoplasmic mass. Frequently they appear aggregated in rather dense groups in certain regions, while in adjacent regions of the cytoplasm they are less numerous. Spore development now begins with the formation of cleavage furrows, which usually arise first on the external surface of the entire aethalium and cut down at all angles into the homogeneous protoplasm. These furrows are very narrow and sharp in some cases, and quite widely opened in others (*fig. 1*). This latter condition may be due, at least partly, to a slight shrinkage in fixation. Very commonly they are curved and forked so as to cut off a superficial layer of segments. Almost simultaneously with the formation of these furrows on the surface of the entire aethalium, similar furrows are formed on the surfaces of the lacunae of the contracted protoplasmic reticulum as described above. These surfaces, of course, are in reality external surfaces of the protoplasm, and the formation of cleavage furrows from them is not in any sense to

be compared to the cleavage from vacuolar surfaces as I have described it in Pilobolus (**4**).

As noted above, the first cleavage furrows commonly do not cut through the entire mass of protoplasm in which they are formed, but curve and fork so as to cut off one or more superficial layers of segments. Further furrows, not continuous with the first, then cut through the central mass, dividing it up into large blocks, each with many nuclei. Meanwhile the superficial segments have still further divided, so that we regularly have one or few nucleated masses at the surface, while the central protoplasm is relatively undivided. The segmentation is very plainly a progressive process proceeding from the periphery toward the center. There is no such thing as a simultaneous breaking up of the protoplasm into uninucleated fragments. The protoplasm which thus segments is quite homogeneous, as noted above. There is no differentiation of hyaline zones or other specialized regions prior to the formation of the cleavage furrows. Furthermore, the nuclei show no special distribution about the cleavage planes. As seen in *figs. 1* and *2*, it is quite common to find a group of nuclei on one side of a cleavage furrow while they are lacking over a considerable area on the opposite side. There is no indication whatever at this stage that the nuclei exert any direct influence on the orientation of the cleavage planes.

If we examine the protoplasm immediately in front of one of these cleavage furrows also, we find it without differentiation of any sort which would indicate the direction which the furrow will take. Aside from the fact that it is common for the cleavage to advance along the same plane or curve in which it has started, it is quite impossible to predict in the case of any unfinished furrow, *i. e.*, one which has not yet cut through the protoplasm in which it lies, what direction it will take. It is very noticeable that these cleavage furrows do not necessarily cut the mass which is segmenting through its shortest axis, any more than through its middle. It is very common to see a strip or sheet cut off from the side of a larger mass in such a fashion that the plane of cleavage lies in the long axis of the mass which is

divided. I have even found some evidence that sausage-shaped masses are cut out of the center of larger masses by means of a cylindrical cleavage furrow. It is very common to find semi-cylindrical masses cut from the surface of the protoplasm by two furrows which curve toward each other so as to form a trough-shaped cleavage surface. All of the above varieties as to form and direction of the cleavage furrows are illustrated in *figs. 1–4*.

At and immediately prior to the time when cleavage commences in Fuligo, its nuclei are all in the resting condition. None of the nuclei indicated in *fig. 1* were dividing. A very little later, almost simultaneously with the formation of the first superficial cleavage segments, the nuclei throughout the entire aethalium begin to divide karyokinetically. In some cases it may be that the peripheral nuclei commence to divide earlier than those which lie deeper. But the difference, if it exists, generally is a very slight one. On the other hand, the process of division seems to begin progressively rather than simultaneously in different parts of the aethalium, regardless of depth from the surface. This is shown by the fact that in examining sections different stages of karyokinesis are found in different parts of the same section. For example, all the nuclei in a certain region a few hundredths of a millimeter in diameter may be in the equatorial plate stage. Moving from this region in one direction one will find a gradual transition to the anaphase stages. Moving in another direction one may find prophases, or one may find nuclei in anaphase on all sides of a region showing only equatorial plates. There is no constancy in the order of stages which will be found in moving from the peripheral to the central or deeper portions of a section cut radially to the surface of the oval, cake-shaped aethalium. It is an absolute rule, however, that widely separated stages in division are never found in close proximity to each other, at least in continuous masses of protoplasm; and generally, passing over one of the numerous lacunae, which, as noted above, pierce the aethalium in all directions, does not involve any sudden transition in the

stage of nuclear division. We may assume that the nuclei begin to divide at numerous isolated points in the aethalium, and that the nuclei of adjacent regions begin their division progressively in all or only in certain directions. If we consider that the division begins in response to a stimulus either external or internal, we should imagine the stimulus being propagated in one or several directions, from the point of its first effectiveness. Strasburger has remarked upon this same wave-like progress of the tendency to nuclear division in Trichia, and has compared the phenomena there with those in the division of the nuclei in the young endosperm of Fritillaria, in which the nuclei at one end of the embryo sac begin to divide first, and the process is then taken up progressively by the successive nuclei through the whole length of the endosperm layer.

Nuclear division proceeds thus during the whole process of cleavage, but without any relation whatever to the latter process. Karyokinetic figures can be found oriented in all possible ways to the cleavage furrows described above, and any stage in division can be found in segments of the dividing cytoplasm of any shape or size, as will be seen from *figs. 4–9*. As a further example, segments can be found with a single nucleus in any stage of division (*figs. 11, 13, 15, 16*). The details of these nuclear divisions, so far as I have been able to work them out, will be described below.

The time relations of nuclear and cell division in Fuligo are thus seen to be entirely different from those in Trichia as described by Strasburger. In Trichia nuclear division is complete before cell division begins, while in Fuligo the two processes are carried on simultaneously. The difference may be associated with a necessity for more rapid ripening of the fruit body of Fuligo. According to my observations, the building of the aethalium and formation of the spores takes place within twenty-four hours in the case of Fuligo. Strasburger does not state just how long a time is required for the development of the sporange of Trichia, but Lister found that they require from two to four days to ripen after their first appearance.

We may return now to the further consideration of the cleavage processes by which the aethalium is cut up into spores. We have noted above that the primary cleavage furrows cut into the surface of the protoplasmic mass at varying angles, that they may be curved, may branch, etc., in the most irregular fashion, with no reference whatever to the distribution of the nuclei. This much of regularity, however, can be seen. The furrows are so oriented with reference to each other and to the surface of the mass that cleavage at first progresses more rapidly at the surface than in the center. Thus, one or more layers of very irregular one- to several-nucleated segments are cut off on the periphery, while the central mass has been cut through by only a few furrows. What is true of the exterior of the aethalium as a whole is also true of the surfaces of the interprotoplasmic gaps or lacunae. It can be seen from *fig. 2* that the surface of each such lacuna is lined by a layer of one- or few-nucleated segments, while beneath them larger multinucleated segments are found. The peripheral segments are very irregular in shape, as are also the larger central segments. Frequently broad thin plates are found; elongated sausage-shaped masses are also common. As noted above, the cleavage planes follow no such simple rules as cutting through the short axis of the mass to be divided, or always dividing a mass successively in planes that intersect at right angles. Hofmeister's law, also, that cell division always occurs transversely to the axis of most vigorous growth, has no application here, since no growth is taking place at the time when these divisions occur.

If we study the cleavage of any one of these central masses of protoplasm we shall find the orientation of the furrows essentially similar to that of those which cut in from the surface of the entire mass. *Fig. 5* shows such a mass with its nuclei all in the equatorial plate stage, both polar and profile views of the latter being shown. It is to be especially noted also that the furrows in *fig. 5* are not directly continuous with those that appeared first on the surface of the mass from which the segment in question was taken. No single furrow can be traced

for any great distance in an unbroken plane or curve. The furrows which are to further subdivide the segment represented in *fig. 5* have been formed independently of those by which the block itself was delimited. The plan seems to be that each furrow may cut through the mass in which it originates, but may not continue across the furrows with which it intersects so as to cut through successive masses in any specific direction. There are no general planes of cleavage for the whole mass. As noted already, the first furrows that form do not as a rule cut deep down into the mass toward a center. Rather they branch or are curved so as to cut off irregular blocks on the surface. New furrows forming on the surface of these, and at very varying angles with them, continue the cleavage into the deeper portions of the mass.

It is well shown in *fig. 5* that the furrows at this stage also cut into a perfectly undifferentiated mass of protoplasm, there being absolutely nothing by which to predict the path they will take except the general direction which they have already entered upon. The protoplasm is singularly homogeneous, without large vacuoles or inclusions of any sort, and through this undifferentiated mass these furrows are formed. It is further seen that they may be either plane or curved, and lie at various angles with each other and the surface of the mass.

This type of cleavage results in no very definite conditions as to the size of the segments formed. Still we can find a stage when the peripheral segments of the mass are quite regularly uninucleated and the deeper portions have been cut to various dimensions, each containing from eight to sixteen nuclei. So far the process resembles that already described for Synchitrium; but at this stage a very noticeable difference in the method of division makes its appearance. This difference is shown in *figs. 6–9*. Whereas, hitherto, there has been absolutely no differentiation of the protoplasm to mark the path to be taken by the cleavage furrows, now broad hyaline areas are formed midway between each pair of dividing nuclei. These are not at all hyaline zones of equal thickness, but furrows broader at the surface

and narrowing toward the centers between each pair of nuclei. The surface is generally slightly depressed in the line of these hyaline regions, indicating the beginning of the furrow which is actually to sever the portions thus preliminarily marked off by the hyaline regions.

The appearance is as if all the denser portions of the protoplasmic mass had contracted about each nucleus as a center, thus leaving irregular, furrow-shaped, less dense spaces in the middle region between each pair of nuclei. These areas contain very little or no stainable material, and seem to be filled with a watery liquid merely. They are, however, not in any sense rounded vacuoles whose cell sap shows surface tension where it comes in contact with the denser protoplasm. The surface of the rounded mass of protoplasm aggregated about each nucleus is by no means smooth and even, as is the surface of the protoplasm about a vacuole. The denser protoplasm passes over by insensible gradations into the less dense material in a fashion very hard to reproduce in a drawing. Peripherally these hyaline areas are bounded by a very thin protoplasmic film consisting of little more than the plasma membrane itself, which can here be more perfectly recognized as a distinct membranous film than in any other condition of cell development which I have yet observed. The plasma membrane is in these stages never broken through. It always forms a perfectly continuous enveloping layer surrounding the entire segment which is being divided, as is shown in *figs. 6–9*. In addition to the existence of these hyaline areas which predetermine the future cleavage planes, another striking condition is to be noticed. The hyaline regions in Fuligo bound off in every case a single nucleus and never a group of nuclei. This nucleus may be in process of dividing, but the daughter nuclei are never completely reconstructed at a stage when these hyaline regions are present cutting off the pair. The segregation is about the nuclei as units, and the cleavage thus predetermined is to be a cleavage by which the entire mass will be cut into uninucleated segments. Whereas hitherto the cleavage planes in these central masses have been

oriented without especial reference to the distribution of the nuclei, thus cutting off larger and smaller segments with more or fewer nuclei, they are now to proceed midway between each pair of nuclei, so that equal and uninucleated masses will result. As already noted, the impression is very strongly given by these dense rounded masses separated by relatively watery regions that a contraction has taken place about each nucleus as a center. Such conditions might be produced by a contraction originating in the structure of the cytoplasm itself, or in a pull exerted upon the cytoplasm by the nucleus. After the formation of these hyaline areas, the cleavage is completed by the furrowing of the plasma membrane along the lines marked out. A later stage than that shown in *fig. 6*, in which certain furrows have already cut deeply down into the hyaline regions, is shown in *fig. 7*. The furrows are apparently formed just as they were in the earlier stages, but they follow the hyaline areas, and thus the separation of the uninucleated masses is completed. Such cleavage as this is progressive in the sense that both the hyaline regions and the constriction furrows are developed gradually from the surface inward. When complete, however, it results in the simultaneous production of a number of uninucleated cells equal to the number of nuclei in the original mass. And in this respect it differs markedly from the cleavage in the earlier stages, in which larger multinucleated masses are progressively cut up into segments with fewer and fewer nuclei. I have already figured a similar differentiation of a hyaline region predetermining the plane of cleavage in the formation of the spores of Pilobolus (**4**, *pl. 25, fig. 21*). In this figure resting nuclei are shown in two groups, between which a less dense zone is formed, through which later, as is shown in *fig. 22*, a cleavage furrow passes. The appearance was little regarded in my description of cleavage in Pilobolus, but its appearance at a similar late stage in the cleavage of Fuligo indicates that it may have considerable significance in connection with the relations of the nuclei to the cleavage phenomena. The hyaline areas, in Fuligo at least, appear at about the stage in cleavage when the furrows

seem to be more definitely oriented with reference to the distribution of the nuclei. At the stage of embryonic growth, when they are found in Pilobolus also, cell division and nuclear division are proceeding in a somewhat definitely correlated fashion. It can hardly be questioned that whereas in earlier stages the cleavage was largely independent of the nuclei, it comes later to be directed solely with reference to their distribution, and it seems not unnatural to assume that in this latter stage the nuclei control the orientation of the cleavage planes. If this is the case, it is quite possible that the formation of the hyaline zones is the visible expression of this activity of the nuclei.

On the other hand, it is quite possible to assume that cleavage throughout is controlled by the cytoplasm, at first with little reference to the distribution of the nuclei, but later with special reference to the formation of uninucleated cells. The formation of hyaline zones preceding the cleavage furrows might in this case also mark the transition from the earlier irregular to the later more definitive stage of cleavage without implying any special activity of the nuclei. I have already noted that it is quite as easy to assume that the cytoplasm itself contracts about the nuclei as that it is drawn together by a tension exerted from the nuclei. Either view is consistent with the assumption that material for the growth of the plasma membranes is formed in the nucleus and passes outward from it to the newly-forming cell boundaries. As is seen from *figs. 6–9*, the nuclei are dividing while the cleavage just described is going on, so that the uninucleated segments formed become almost immediately binucleated. Cell division then follows either before or after the complete reconstruction of the daughter nuclei (*figs. 16, 17*). Thus, in the end, uninucleated spores are produced. The formation of a hyaline region and constriction furrow for the division of a binucleated cell whose nuclei are already in the anaphase stage is shown in *fig. 9*. The beginning of the constriction for the final division of a binucleated cell to form two uninucleated spores, the hyaline region having not yet appeared, is shown in *fig. 16*.

Transition types of cleavage between that without and that with a preliminary formation of a hyaline furrow are abundant. *Fig. 8* shows a six-nucleated mass of protoplasm dividing by furrows, which in three cases are preceded by hyaline differentiation, and in the other two cases are cutting directly into the undifferentiated protoplasm.

With the formation of uninucleated segments whose nuclei divide no more, the process of cleavage is complete. As noted above, the cleavage results in uninucleated segments at the periphery of the protoplasmic masses much earlier than in their interior. The nuclei in these early formed segments are always found dividing. The definite spore cells with a single resting nucleus are probably formed first in those regions of the aethalium where cleavage first began. The final delimitation of the spores seems to proceed progressively from these regions in all directions through the aethalium. Fully formed spores may ultimately be found throughout the greater part of the aethalium, while in certain regions here and there cleavage may still be in progress. There is, however, in the later stages of cleavage no such marked difference between peripheral and central regions as there was during the early stages. Whether this is due to retardation in the cleavage at the periphery during the later stages, or whether the nuclei there divide repeatedly to prolong the process, I have not been able to determine.

Summarizing, we may characterize the whole process as one of progressive cleavage by means of furrows which cut through the protoplasmic mass in very many directions and at very varying angles to each other. The process is progressive both in that the furrows originate on the surface and proceed gradually toward the center, and in that larger multinucleated segments are first formed which are by further divisions reduced to the condition of uninucleated spores. It may perhaps be distinguished from bipartition as a process of successive multipartition, since cleavage furrows may invade any particular portion of protoplasm simultaneously from a number of directions. At first the orientation of the cleavage planes shows no evident relation

to the distribution of the nuclei. Later the furrows proceed in every case so as to cut off uninucleated masses approximately equal in size. This later period of cleavage is characterized in many cases by the aggregation and rounding up of the denser cytoplasm about the nuclei so as to leave hyaline regions midway between each pair of nuclei, thus predetermining in each case the plane of cleavage to be followed later by the cleavage furrow. This type of cleavage results immediately in every case in the formation of uninucleated cells whose nuclei, however, may still be in a state of division. In the end, the entire protoplasm of the aethalium has been cut into uninucleated segments which are at first naked bits of protoplasm. Later each cell becomes surrounded by a wall and constitutes a spore.

Turning now to the phenomena of nuclear division, we may note first of all that the structure of the resting nucleus conforms, in spite of its small size, to that of the nuclei of other fungi and the higher plants. Nuclear membrane, chromatin (nuclein), and nucleole are present, and are differentiated by staining with safranin, gentian-violet and orange, just as sharply as they are in the pollen mother cells of the lily. The nucleole frequently lies in a clear space (*fig.* 10), as is so frequently seen in the nuclei of the root-tip of the onion.

The nuclei, however, are too minute for the successful study of the prophases in spindle formation. In the equatorial plate stage (*figs.* 3, 5, 11) the spindle is sharply differentiated. It shows rather sharp-pointed poles which may be more densely stained at their tips. Broad-poled spindles, such as those figured by Strasburger for Trichia, are not found in Fuligo. The chromosomes stain deeply in the equatorial plate stage. In polar views it is possible to count the number with considerable definiteness. The great number of these figures to be found in sections of Fuligo at this stage make the material especially favorable for such study. The chromosomes are relatively short and thick, and form a very regular equatorial plate, all of them lying practically in a single plane, so that in polar views they are practically all in focus at once. From a study of a large number of

such figures, I am quite certain that the number of chromosomes is twelve.

All stages of the separation of the daughter chromosomes and their migration to the poles of the spindle can be found in the greatest abundance (*figs. 6, 8, 12–15*). The spindle becomes slightly elongated during this process. Connecting fibers are present and form a figure closely resembling that in the corresponding stages in the lily or larch. As the chromosomes first separate, relatively large gaps are seen between the connecting fibers, which appear bunched together in a few large strands (*fig. 13*). The whole connecting spindle is markedly barrel-shaped at this stage. As the chromosomes approach the poles, the connecting fibers become more evenly distributed, and are straightened so as to form a cylindrical series extending between the groups of daughter chromosomes (*fig. 14*). A marked difference between the nuclear divisions in Fuligo and those in the asci I have studied is seen in the arrangement of the daughter chromosomes as they are drawn back to the poles. In the asci these chromosomes are widely scattered on the spindle at this stage, some having nearly reached the poles, while others are much nearer the equatorial region. This condition makes this stage the most favorable for counting the chromosomes of the nuclei in the ascus. In Fuligo, on the other hand, all the daughter chromosomes retreat simultaneously toward the poles, as is seen in *figs. 6, 13, 14*. They also become quite densely massed together, so that the individual chromosomes are not so easily distinguished in polar views at this stage as in the equatorial plate stage.

As the daughter chromosomes reach the poles, the whole figure is still more elongated, the connecting fibers being drawn out (*fig. 15*) into a long slender strand, which gradually disappears. The poles of the spindle can be distinguished beyond the groups of daughter chromosomes till a very late stage (*figs. 9, 15*).

The nucleole of the parent nucleus in Fuligo disappears at a rather early stage as compared with other fungus nuclei. It is

never to be found lying midway between the daughter nuclei near the old spindle, as is regularly the case in many asci. The daughter nuclei are apparently reconstructed in the ordinary fashion. The figures, however, are too small to show very characteristic details at this stage.

The similarity of the whole process of nuclear division in its main outlines here to what is found in higher organisms is certainly very striking, and shows clearly enough that simplicity of structure and life history on the part of the whole organism is by no means to be taken as indicating a corresponding reduction in the complexity of the nuclear structures and activities.

The capacity of the slime molds to become encysted at any stage in their life history when conditions become unfavorable is very well known. A condition which I have sometimes found, and which is represented in *fig. 19*, indicates that this may occur midway in the process of cleavage. The aethalium in question was made up of rounded, two- to several-nucleated masses, each provided with rather a thick wall. Whether later with a return of favorable conditions such masses would continue their cleavage, and form normal uninucleated spores, or whether they would themselves function as spores, I have not been able to determine. A normal uninucleated spore is shown in *fig. 18*.

The aethalium and the sporanges of the Myxomycetes differ from the sporanges of Synchitrium, Pilobolus, and Sporodinia, whose method of spore formation I have already described (**4**), in that the multinucleated condition in the former originates at least in the formation of the plasmodium. The plasmodium is a product of cell fusions without nuclear fusions, so far as known at present. Physiologically considered, in all its functions of nutrition, growth, and response to external stimuli, it is the equivalent of such multinucleated masses of protoplasm as are formed simply by growth and nuclear division without cell division. The plasmodium itself increases its original volume, as formed by fusion, by this same type of growth. Fundamentally considered, it is the physiological equivalent of the multinucleated mass formed in Synchitrium by the division of the original

single nucleus of the vegetative body. The internal relations of nuclei and cytoplasm cannot be conceived as different, merely because in one case the nuclei and separate cytoplasmic masses were brought together by fusion, while in the other they were formed from a uninucleated cell by growth and nuclear division.

Morphologically, however, the two structures must be regarded as entirely distinct, and the possession of the plasmodium, and the capillitium formed as a deposit in vacuoles by the slime molds, is probably sufficient reason for regarding them as constituting a separate developmental series running back to an origin independent of any of the existing groups of algæ or fungi. Sachs (**18**) has quite recently expressed the opinion that they are to be classed with the fungi, but he brings no morphological evidence to support his view. There can be no question that the Acrasieæ represent simpler forms out of which the Myxomycetes have developed, and we thus have a developmental series leading from simpler to more complex forms. The plasmodium and capillitium, appearing only in the more specialized members of the group, are plainly secondarily acquired structures developed as additions to the structural features of the Acrasieæ, and are not to be directly homologized with physiologically equivalent structures in other groups.

The physiological equivalence of the plasmodium and the multinucleated masses of protoplasm found in other fungi, such as Synchitrium, Pilobolus, etc., can hardly be questioned. Elsewhere (**3**) I have discussed the question as to whether these multinucleated masses should be classed as single cells, or as the equivalents of many-celled tissues or organisms. The plasmodium of a slime mold is well calculated to furnish further evidence on this point. In its method of origin by the fusion of distinct amoeboid swarm-spores, it would seem to testify to its multiple nature; still, as noted above, the whole physiology of the plasmodium in its nutrition, reactions to stimuli, and growth, shows most conclusively that it is a unit in exactly the same sense as is an amoeba, or one of the swarm-spores which combined to form it. In fusing, the swarm-spores gave up their individuality

to become parts of a larger mass. That this fusion did not involve a fusion of their nuclei cannot be considered as altering the result so far as the question of individuality is concerned. Where sexual cells fuse and their nuclei unite there is no question that the resulting fertilized egg is a single cell. If, as Häcker (2) has shown is the case in Cyclops, the pronuclei remain distinct through the early cleavage stages of the egg, this cannot be taken as evidence that the two nucleated bodies thus produced are not single cells rather than the equivalents of tissues. These binucleated cells, functionally and morphologically considered, are the equivalents of the later cells of the Cyclops which appear with a single nucleus. The conclusion must be, as I have already pointed out, that the individuality of the cell is independent of the number of nuclei which it contains.

Hertwig argues for the *potential* equivalence of multinucleated cells and tissues. The word potential here of course may mean much or little. In support of his view he urges the case of the insect egg, whose nucleus divides to form hundreds of daughter nuclei before cleavage begins. Later the multinucleated yolk mass is by cell division separated into a blastoderm of as many cells as there were nuclei present. It is quite plain, says Hertwig, that the apparently simple egg could not with a single stroke, as it were, have become a multicellular organism. The question here, of course, is how great a change is involved in the transition from the one-celled to the many-celled condition, and on this point it is interesting to note that up to the stage when cell division takes place in the insect egg there has been no visible differentiation of embryonic structures in the egg. The cel division simply transforms the one cell into a mass of equivalent cells, and this need hardly be considered as a change too great to be due entirely to the cleavage process. The relation of multinucleated and uninucleated cells is well shown in the very fact that the visible differentiation of the insect embryo, aside perhaps from the determination of its axes, which was accomplished even earlier, begins after the division of the egg into numerous cells, and not while it remains a single cell, although

it has meanwhile become multinucleated. It would seem that differentiation was dependent to a certain degree, at least, on the interaction of individualized protoplasmic units, each capable of receiving and reacting to independent stimuli as the parts of a multinucleated cell cannot. Hertwig, in his doctrine of biogenesis, himself insists on the importance of the interaction of separate cells for the production of physiological differentiation and division of labor.

To be sure, we have abundant evidence that the multinucleated cell can achieve a certain degree of differentiation, as is shown in the numerous Siphoneæ which mimic in their root-like, leaf-like, and stem-like structures, the analogous parts of the higher plants. It is perfectly apparent, however, that this differentiation is on a far simpler scale than is seen in the complex mechanical and other tissue systems and organs of the higher plants. Indeed, the relative unimportance of the Siphoneæ as a part of the earth's vegetation is to be regarded as very strong evidence that the type of structure which they show in their multinucleated cells is by no means well adapted to develop complexity and differentiation of structure such as is necessary to meet the manifold variations in environmental conditions to which all plants are subjected. These Siphoneæ are after all hardly more differentiated than the infusorians, which are typically unicellular. Pfeffer (**16**) puts the case very strongly when he points out that we can conceive of no such independent units in the multinucleated cell as the energids of Sachs are defined to be. If the energid is a nucleus with a portion of cytoplasm under its immediate control, there can be no such structures in the Siphoneæ, since the protoplasm of their cells is constantly streaming from one point to another, with the exception of the plasma membrane, which remains fixed. No nucleus could thus have any definite relations with any particular portion of the plasma membrane, and it is hard to conceive that in this streaming motion any portion of the semifluid cytoplasm should remain constantly in connection with any particular nucleus. As Pfeffer says, we must conclude that any specific mass of cytoplasm in a

multinucleated cell will be simultaneously influenced by various nuclei which are in contact with it. There can be no invisibly bounded units in which the same living substance remains united. Pfeffer also justly objects to Sachs's characterization of the Siphoneæ as *noncellular* plants, and regards them as both morphological nnd physiological units.

If we compare the method of spore formation in Fuligo with that which I have described elsewhere (4) for Synchitrium, Pilobolus, and Sporodinia, it will be seen that the processes in all these forms are identical in their main features, while differing in a number of important details. In the four cases the cleavage is progressive from the surface inward, larger segments being first formed, which are later cut up into uninucleated cells, except in *Synchitrium taraxaci* and Sporodinia, in which the multinucleated segments function directly as spores.

In the earlier stages of cleavage in Pilobolus and Fuligo the furrows pierce through perfectly undifferentiated and quite homogeneous protoplasm, while in the later stages the differentiation of hyaline areas, wedge-shaped in transverse section and cutting through the masses to be divided, predetermine the planes of the cleavage furrows. Such hyaline areas were not observed in Synchitrium or Sporodinia. In Synchitrium and Sporodinia nuclear divisions precede cleavage. In Pilobolus nuclear divisions occur during the later stages of cleavage, and in Fuligo nuclear divisions and cleavage proceed simultaneously throughout.

Fuligo is the only one of the five forms in which the uninucleated segments formed by the completion of the cleavage process, and which I have called protospores, become the functional spores directly without further growth or nuclear division. In this respect perhaps the cleavage of Fuligo represents a more simple primitive type than that of either of the others.

In all forms the orientation of the furrows with reference to the surface of the dividing mass and with reference to each other is extremely varied, and it can be laid down as a general rule for the forms studied that no one furrow can be traced

continuously through the entire sporange or aethalium which is to be divided except, perhaps, in the case of the very thin layer of spore plasm in Sporodinia. On the contrary, by the curving and branching of the furrows, segments irregular in their size, shape, and number of nuclei are cut off successively from the periphery toward the center. These segments in turn, and also progressively from periphery to center, are cut up by new furrows into smaller segments, until finally in *Synchitrium decipiens*, Pilobolus, and Fuligo the uninucleated condition is reached. No general system of cleavage planes, either parallel or radial to the surface of the dividing mass, can be discovered. The path of the cleavage planes as division progresses becomes an inextricable confusion of zigzag lines, branching and intersecting at almost every angle. The occurrence of such similar types of cleavage of the multinucleated mass as are found in the aethalium of Fuligo and the sporanges of the Phycomycetes must be regarded as another example of parallel development in structures not phylogenetically connected. The explanation of the similarity in these forms of cleavage is to be sought in the fundamental physiological properties of protoplasm, and not in hereditary transmission to the different branches of a series of genetically related forms.

With the above account of fusion in Fuligo representing the Myxomycetes, types of all the main groups of fungi producing asexual spores in the interior of mother cells have been described except the Oomycetes, and while it will be necessary to investigate representatives of all the genera, at least, in these groups, still the hypothesis is fairly justified that some form of progressive rather than simultaneous cleavage by cell plates will be found in every case. Klebs's (15) investigations of Hydrodictyon also indicate that the formation of zoospores is at least not by simultaneous division into uninucleated segments, and the whole process in this alga should be further investigated, especially with reference to the occurrence of the nuclear fusions which Klebs describes as occurring in the developing zoospores. It may be noted here also that Bachmann (1), in describing a new species of Mortierella, has observed incidentally the marking off of the

surface of the sporange into irregular polygonal areas at the time when spore formation is beginning. The lines marking off these areas are doubtless the beginnings of the cleavage furrows as I have described them for Pilobolus and Sporodinia. Bachmann made no sections, but concludes that the spore formation must be a progressive process.

A sufficient number of forms has been investigated to show that progressive cleavage is a widely spread phenomenon among the lower plants, occurring in very many cases when multinucleated masses are to be cut up into smaller cells. That simultaneous cleavage may also occur is quite possible, but the evidence for it is not strong except, perhaps, in the case of the sporange of the Saprolegniaceæ.

I have already shown elsewhere (4) that the progressive cleavage of the sporange in spore formation is in principle the same process as that which has been described as division by constriction in Cladophora and the conidiophores of the mildews, and it will be of interest to attempt a comparison of this progressive cleavage with cell division as found in the growing points of the higher plants, especially from the standpoint of the more general theories of cell division.

Schleiden's (21) doctrine that the form of a plant is determined by its cellular composition, including as the two important factors the arrangement of the new-formed cells in growing regions and the subsequent varying growth and enlargement of these cells in their three dimensions, was first opposed by Hofmeister. Hofmeister (7 and 8, p. 129) advanced the view that cell-formation is subordinate to the growth of an enlarging organ taken as a whole. He held that the growth of the single cells of a vegetative point is controlled and conditioned by some formative principle which determines the growth of the entire organ, this latter being directed toward simple enlargement or the development of some predetermined form. According to this view, growth of the vegetative point cannot be interpreted as determined by the sum of innate growth tendencies of the individual cells.

Hofmeister believed that cell division takes place according to a simple mechanical law. The new cell wall always cuts the axis of most intense growth at right angles (**8**, p. 127). It is plain that such a principle as this can have no application in interpreting the division of the masses of protoplasm in sporanges whose growth is complete, the accompanying tensions, as may be fairly assumed, having also reached a condition of equilibrium.

Sachs (**20** and **18**, p. 22) follows Hofmeister in regarding the growth and division of the single cells as subordinate to the growth of the vegetative point as a whole. From a study of the arrangement of the cells in the growing point of the higher cryptogams and flowering plants, he has developed the law of the rectangular intersection of cleavage planes in the successive cell divisions. He regards this as the most universal law of structure in the plant world, and holds that it is independent of all phylogenetic relations, and not a result of natural selection. As is well known, he holds that the outer form of the growing organ is the primary determining factor for the orientation of the cleavage planes. The periclines conform directly to the surface of the embryonic organ. The anticlines cut the periclines at right angles, and if division is to occur in three dimensions the transversals also appear in a third plane at right angles to the other two. Sachs believes that the relations thus expressed are of so fundamental a nature as to be comparable to those determining the relations of the axes of a crystal. The cellular structure of a young organ is related to these *Leitlinien* as the structure of a crystal to the arrangement of its faces and their intersecting angles. The form of a growing point is determined by phylogeny, and selection when given the direction of the cleavage planes, is at once known. Such fundamental relations as these, occurring in the most widely separated groups, are to be considered as innate in protoplasm. Sachs proposes to call them mechanomorphoses. The principle thus developed by Sachs has been generally accepted as explaining the arrangement of the cell walls in the growing points of the higher plants,

and, as Sachs himself notes, the investigation of the apical cel- and its divisions has ceased to be regarded as affording a key to the explanation of the development of shoots.

Assuming the correctness of Sachs's law for the higher plants, if we attempt now to apply it to the case of the cell-division in sporanges we are confronted with many difficulties.

First of all, these sporanges do not divide by successive bi-partitions as do the cells in the growing points referred to. Nor do they divide by simultaneous delimitation of the energids which, according to Sachs's view, compose them. Their cleavage is progressive, and the cleavage planes, as shown in sections, form no great series comparable to the anticlines and periclines which Sachs finds in a section of a root tip. In the cleavage of the sporange the principle of rectangular intersection is violated constantly, the angles of intersection of the cleavage planes showing no constancy whatever. It may be objected that the sporange is not a growing organ, and hence its method of division should not be expected to conform to that of growing points. Growth is complete in the sporange before division begins, though it may recommence in the later stages of cleavage in Pilobolus. The process in the sporange consists in cutting up into cells a mass of protoplasm whose form has been already determined and its growth completed. Still, although Sachs states the principle of rectangular intersection for growing points, and conceives it as determining the arrangement of the cells in the growing point as it pushes forward in the elongation of the shoot, he always conceives the divisions as occurring in these growing points after the essential embryonic growth of the cell concerned is at an end. Growth of cells subsequent to division may, and generally does, in his opinion, distort the relations of the cleavage planes.

Sachs makes no attempt to include the multinucleated sporangia in his discussion. Still he specifies the growth of the Siphoneæ (**19**, p. 100) as exceptional when compared with the irregular growth of many thallophytes, and considers the development at their growing points as typical of that in the higher

plants. He also specifically states that typical mechanomorphoses are the cell nets in growing points of young organs *and structures whose cells show no individual growth after cell division is complete*. All the sporanges mentioned above would be included in this latter type of structures. The sporange of Synchitrium is a spherical mass of protoplasm which divides into cells which show no further growth, at least till after cleavage is complete. At this stage, then, the cleavage planes should illustrate typical mechanomorphosis. It would seem that in such a spherical mass of undifferentiated protoplasm the opportunity for the law of rectangular intersection to come to full expression would be especially good. We might expect the periclinal and anticlinal cleavage planes to be extremely conspicuous in such a case. On the contrary, as noted above, and as is well shown in *figs. 2, 4, 14, 15, pl. 24*, of my former paper (**4**), no regular periclines and anticlines are to be observed. The surface furrows cut into the mass at very varying angles, frequently also becoming curved and branching so as to intersect near the surface, and thus cut off superficial cell-masses of the most irregular shapes and sizes. There is apparently the greatest irregularity in the orientation of the cleavage planes both with reference to each other and to the surface of the dividing mass, as a glance at the figures referred to above will show, and as I have described more fully in the case of Fuligo. As this method of progressive cleavage, however, is of wide occurrence among the thallophytes, the principle of rectangular intersection loses that universal validity for cell division upon which Sachs so strongly insisted. Sachs uses the law of rectangular intersection so as to further support Hofmeister's doctrine of the subordination of cell division to the growth of organisms as wholes, and in this sense his results have, for the most part, been taken up and utilized for theoretical purposes in discussions on the nature of the cell and its division (see **6** and **26**). With Hofmeister, Sachs considers that the growing point of such a structure as the pine shoot develops simply as a mass of protoplasm analogous to that at the growing end of a Vaucheria filament. The shape of the end

of the shoot has been determined by selection during phylogenetic development; its growth is the enlargement of a protoplasmic mass along lines predetermined. The putting in of the cell walls, being a purely mechanical process following the simple rule of rectangular intersection, can in no way be regarded as determining the shape of the shoot. The tip grows just as does the Vaucheria tip, the formation of individual cells being a purely secondary matter. The independence of the single cells, their growth and division, is entirely subordinated to the growth of the entire organ. It is an open question whether the rectangular intersection of cell walls in vegetative points, assuming that the facts are as Sachs describes them, is sufficient basis for so important a conclusion as that above stated.

Jennings has characterized the law of rectangular intersection as "hardly to be considered as more than a statement of a condition commonly found." There is nothing inherently impossible in the assumption that the habit of forming successive cell plates so that they intersect at right angles has itself been acquired by the cells as individual units. The form of the shoot may then depend on this power of the cells, assuming always a further regulation of their activities by reciprocal stimuli between the cells themselves as well as by stimuli from their environment.

There can be no question that at least the details of cleavage in the sporanges I have studied have been modified in the course of phylogenetic development with especial reference to the needs of the organisms concerned. I have elsewhere pointed out the correlation between the abbreviated cleavage process in Sporodinia and its more rapid spore development. The participation of vacuoles in the formation of the cleavage furrows in Pilobolus is another example of such modifications. That the cleavage processes are after all so similar in the different sporanges studied is doubtless due to the fundamental physical and chemical properties of the protoplasm, but the process is none the less to be conceived of as modified by selection. It

must also be borne in mind constantly that in such cleavage phenomena as these the cleavage planes are influenced in no way by the need of forming any specific tissue or plant form In the growing point of a metaphyte it may perhaps be difficult to decide whether the planes of division are determined by the cells themselves in accordance with internal conditions, or whether, as Sachs claims, it is the shape and differentiation of the shoot taken as a whole which determines the planes of division. In the division of these spore-forming masses no such question can arise, since no differentiated tissue is to be formed. The problem is simply to divide the large mass into smaller masses more convenient for distribution. In doing this, as I have shown, entire irregularity prevails as to the orientation of the cleavage planes with reference to each other, and with reference to the axes of the mass to be divided. It may be concluded that the protoplasm is *per se* perfectly isotropic so far as cleavage is concerned, and that it is a matter of indifference whether the cleavage planes intersect at right angles. If rectangular intersection is the rule in the higher plants, it might well be argued that this is a secondarily acquired condition, assuming with Pfeffer that these multinucleated structures are single cells.

The question can hardly be raised in this connection whether the organism or the cell forms the new cells or spores. Still it is interesting and significant to note, as I have pointed out already, that the process of cleavage in these sporanges is essentially similar in principle to the typical cell division by constriction, which I have described as producing the one-nucleated cells at the base of the conidiophore in the mildews, and which is also found in Cladophora. Progressive cleavage by surface furrows is only a more complex modification of cell division by constriction. Spore formation is simply cell division, though not by repeated bipartitions of the mother cell, as it commonly occurs in the vegetative growth of the higher plants.

The growth of the Multinucleatae cannot then be regarded as illustrating the growth of an organism comparable morphologically to a metaphyte but without cell formation. The

cœnocyte is a cell, and its growth and differentiation is comparable to the growth and differentiation of a single cell in the growing point of one of the higher plants. That such a cell may become multinucleated is illustrated by the multinucleated cells in the red algæ and the striped muscle fibers of animals Just as in these cases, the cœnocyte everywhere is a cell which has bcome multinucleated strictly for functional purposes requiring the distribution of the nuclear material through the enlarged cell body.

The more modern theories as to the division of the animal cell all assume a definite correlation between nuclear and cell division. The mechanism of the one is definitely connected with that of the other. The division of the cell at right angles to the long axis of the karyokinetic spindle is the rule among the higher animals as in the higher plants, and the later theories have assumed this relation as fundamental. Thus, Heidenhain's (**5**) theory assumes that division of the cell is a result of tensions in the unequally stretched elements of a "system of organic rays" extending from the centrosome to the plasma membrane. Kostanecki (**11**) attributes cell division to the development of a cell plate which is formed as a result of the migration of the ends of pairs of polar rays, produced by the splitting of parent rays, from the points of their original attachment to the plasma membrane of the mother cell into the equatorial region between the daughter nuclei. Jennings (**10**) has summarized the theories of cell division and of the orientation of the successive cleavage planes in animal cells, and I need not enumerate them here. They are for the most part directed especially to the explanation of cases where nuclear and cell division have become definitely correlated, and in many cases they assume the determination of the plane of cell division by the axis of the nuclear spindle. Cases of cell division between resting nuclei, such as are found in the cleavage of the insect egg, have received less attention. Still, in all the sporanges studied it is plain beyond all else that nuclear division neither determines nor is in any way connected with cell division, and it is thus shown that the

cytoplasm can divide without any reference to the division of the nuclei. The cytoplasm both initiates and completes the process of dividing any particular cell mass, while the nuclei are throughout in the resting condition in Synchitrium; and, on the other hand, in Fuligo it can carry on the cleavage in essentially the same fashion simultaneously with nuclear division, without apparently being influenced in the least degree by the occurrence of the latter. In Fuligo the axes of the nuclear spindles and the planes of the cleavage furrows may be inclined at all imaginable angles to each other (*figs. 5–7, 8, 9*). It is always to be noted in these cases that the cleavage furrow in question is not the one destined to separate the daughter nuclei which are in process of formation at the time when the furrow is forming.

The cleavage always lags behind nuclear division, never separating any two daughter nuclei until they have reached at least the resting condition, or are themselves engaged in the next following nuclear division. This latter case, as found in Fuligo, is especially interesting as showing most clearly that the apparatus of nuclear and cell division is entirely distinct. The mechanism of nuclear division is in full operation while cleavage furrows are also forming in adjacent regions of the cytoplasm in entire independence of it. It is thus shown that no such conditions exist here as in endosperm formation in the lilies, where, after nuclear division is complete, a set of new connecting spindles are formed between the resting daughter cells. The mechanism of cell division in the endosperm is the same as in ordinary cell division, which follows at once upon nuclear division. It is presumptively the mechanism of nuclear division which is in operation in this simultaneous cell division, though operating independently of and subsequently to the completion of a long series of nuclear divisions. In endosperm formation we find no cases in which the nuclei are themselves dividing at the same time that cell plates are forming between them and adjacent nuclei which are also dividing. On the contrary, I have observed considerable evidence in the division of the pollen mother cells of the larch, in cases where the first nuclear division is not to be followed by

cell division, that the fibers of the connecting spindle are utilized, at least partially, in the formation of the spindles for the second nuclear division.

It is by no means to be argued from the fact that cell division and nuclear division are independent in these simple forms of plant life that the processes are not most intimately connected in the higher plants and animals, nor that the position of the nuclear spindle may not determine absolutely the plane of cleavage in these latter cases. In the higher plants there can be no doubt that the spindle, persisting after nuclear division, forms a cell plate and determines the plane of cleavage. On the other hand, the process of cell division in Fuligo shows very clearly that such a correlation is by no means fundamental or universal. Whether or not the nucleus in any fashion influences the orientation of the cleavage planes in this latter case, it does not do it by means of the karyokinetic spindle.

Wille (**25**) in a preliminary communication has reported the discovery of a type of division in multinucleated cells in which the nuclei participate in the formation of new cross walls. Just what the nature of the nuclear activity is in this case is not clear from the brief report referred to. It certainly represents a new and most interesting condition in multinucleated cells.

It is quite possible that the irregular cleavage of the fungus sporange indicates a primitive condition when nuclear and cell division are entirely independent processes, and that the correlation of the two has been gradually achieved in the evolution of the higher plants and animals. It is plain, therefore, that the theories of Heidenhain and Kostanecki (**5** and **11**) can have no application in the explanation of cleavage in these sporanges, and it is further plain that no theory which interprets cell division as a function of the karyokinetic figure can claim to be of fundamental value for the explanation of the process. Cell division may be much more definitely related to purposeful results in tissue formation when, as in the higher plants, it is accomplished by the mechanism of the spindle and cell plate. It is quite possible that the regular orientation of successive cleavage planes

so as to form tissues, *i. e.*, aggregates of cells with specific shapes and dimensions, only became possible after cell division came to be a function of the same mechanism which effects the division of the nucleus, but, as Strasburger (**22**) long ago pointed out, the process itself is by no means necessarily dependent upon the existence of this particular mechanism or of any correlation whatever with nuclear division.

Hofmeister (**8**) calls attention to the fact that the division of protoplasmic masses for the formation of reproductive cells is quite universally accompanied by loss of water and reduction of volume with increased density of the protoplasm. This phenomenon of contraction and loss of water is especially conspicuous in the process of spore formation in sporanges, as I have already noted. It seems a fairly natural assumption that the tensions set up in a mass of protoplasm which is contracting as a result of loss of water may be utilized in some fashion to produce the extremely irregular cleavage furrows which we observe in the early stages of spore formation. That these furrows, however, are not purely mechanical in their origin and analogous to the fissures that appear on the surface of a drying colloidal mass is shown, as I have noted elsewhere, by the fact that, although irregular, they never cut off segments containing no nuclei, and ultimately they produce approximately equal uninucleated spores. Some form of organization must be assumed to be present in the protoplasm which determines the progress of the cleavage so as to lead to a constant result. Still, even with this assumption, the possibility remains that the source of the energy which is thus controlled may be in the tensions produced by contraction due to loss of water.

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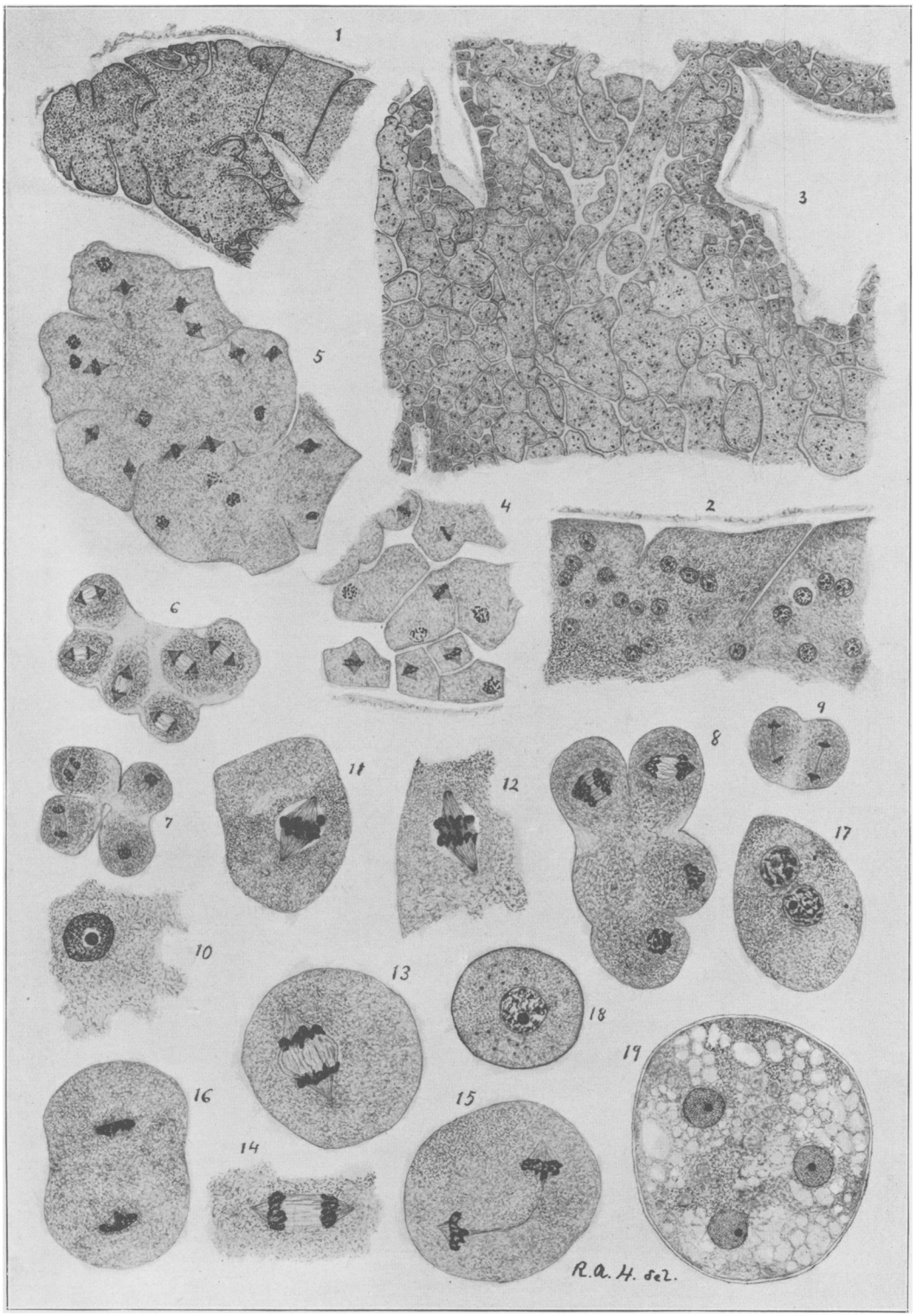

HARPER on FULIGO

EXPLANATION OF PLATE XIV.

All figures were drawn with the aid of the Abbé camera lucida, and all but *1* and *3* with the Zeiss apochr. obj. 2^{mm}. *Figs. 2–7*, and *9* with oc. 6; *fig. 8* with oc. 12; and *figs. 10–18* with oc. 18.

FIG. 1. Portion of aethalium showing first cleavage furrows and lacunae of the protoplasmic reticulum. × about 150.

FIG. 2. Superficial portion of protoplasm showing two cleavage furrows and scattered nuclei.

FIG. 3. Portion of dividing protoplasm from interior of aethalium and lying between three lacunae marked *l*. × about 250.

FIG. 4. Several entire segments and portions of others from surface of lacuna in the interior of an aethalium; profile and polar views of nuclei in the equatorial plate stage.

FIG. 5. Segments from interior of aethalium with cleavage furrows cutting into it from various directions; nuclei in equatorial plate stage.

FIG. 6. Segment cut through by hyaline areas which predetermine the course of the cleavage furrows.

FIG. 7. Later stage in cleavage of a segment like that shown in *fig. 6*.

FIG. 8. Segment showing cleavage furrows cutting through undifferentiated protoplasm and others cutting into hyaline areas.

FIG. 9. Binucleated segment, the nuclei in a late stage of division and separated by a hyaline zone.

FIG. 10. Resting nucleus.

FIG. 11. Uninucleated segment; nucleus in equatorial plate stage; nucleole and part of nuclear membrane of parent nucleus still present.

FIG. 12. Metaphase; part of nuclear membrane of parent nucleus still present.

FIG. 13. Uninucleated segment; nucleus in later metaphase, connecting fibers curved.

FIG. 14. Later stage; connecting fibers straightened.

FIG. 15. Diaster; connecting fibers a narrow strand.

FIG. 16. Dispirem; cell division beginning.

FIG. 17. Binucleated segment; cell division not yet begun.

FIG. 18. Uninucleated spore with thin wall; granules of reserve material in cytoplasm.

FIG. 19. Encysted segment.